

GCSF-03-2004-B.ST25  
SEQUENCE LISTING

<110> Sun, Lee-Hwei K  
Sun, Bill  
Sun, Cecily R

<120> Fc fusion proteins of human granulocyte colony-stimulating factor with increased biological activities

<130> 03SUN2001-B

<140> to be assigned

<141> 2004-03-15

<150> US 09/968362

<151> 2001-10-01

<160> 28

<170> PatentIn version 3.1

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<211> 30

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<213> Artificial Sequence

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<220>

<223> PCR primer

<400> 3

gagcgcaaatt gttgtgtcga

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<210> 4

<211> 28

<212> DNA

<213> Artificial Sequence

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<223> PCR primer

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28

<210> 5

<211> 29

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<213> Artificial Sequence

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<223> PCR primer

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29

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ttgtgtcga

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<213> Artificial Sequence

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<223> PCR primer

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gagtccaaat atggtccccc a

21

<210> 9

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 9

ggaattctca ttaccacaga gacagga

28

<210> 10

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<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 10

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21

<210> 11

<211> 60

<212> DNA

<213> Artificial Sequence

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<223> PCR primer

<400> 11

gagtcctaat atggtcccc atgcccacca tgcccagcac ctgagttcgc ggggggacca

60

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<212> DNA

<213> Artificial Sequence

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&lt;210&gt; 16

&lt;211&gt; 70

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; PCR primer

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 cacatgcccc 70

&lt;210&gt; 17

&lt;211&gt; 1368

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; hG-CSF-L-vFc gamma2 (Figure 2A)

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 ggcgatggcg cagcgtccca ggagaagctg tgtgccacct acaagctgtg ccaccccgag 240  
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 agccaggccc tgcagctggc aggctgcttg agccaactcc atagcggcct tttcctctac 360  
 caggggctcc tgcaggccct ggaagggatc tcccccgagt tgggtccac cttggacaca 420  
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 cgccgggcag gaggggtcct agttgcctcc catctgcaga gcttcctgga ggtgtcgtac 600  
 cgcgttctac gccaccttgc ccagcccga tccggtggcg gttccggtgg aggcggaagc 660  
 ggcggtggag gatcagagcg caaatgttgt gtcgagtgcc caccgtgccc agcaccacct 720  
 gtggcaggac cgtcagtctt cctcttcccc caaaaccca aggacaccct catgatctcc 780  
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<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<223> hG-CSF-L-VFc gamma2 with a 30-amino acid leader peptide (Figure 2A)

<400> 18

Met Ala Gly Pro Ala Thr Gln Ser Pro Met Lys Leu Met Ala Leu Gln  
1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro  
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu  
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys  
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu  
65 70 75 80

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser  
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu  
100 105 110

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Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu  
115 120 125

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala  
130 135 140

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu  
145 150 155 160

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg  
165 170 175

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu  
180 185 190

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly  
195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Arg Lys Cys  
210 215 220

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser  
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val  
290 295 300

Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Ser Ile Glu Lys Thr  
325 330 335

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
340 345 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
355 360 365



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Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp  
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

<210> 19

<211> 1371

<212> DNA

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma4 (Figure 2B)

<400> 19

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gccagctccc tgccccagag cttcctgctc aagtgttag agcaagttag gaagatccag	180
ggcgtatggcg cagcgtcca ggagaagctg tgtgccacct acaagctgtg ccaccccgag	240
gagctggtgc tgctcggaca ctctctgggc atccccctggg ctcccctgag cagctgcccc	300
agccaggccc tgcagctggc aggctgcttg agccaactcc atagcggcct tttcctctac	360
caggggctcc tgcaggccct ggaagggatc tccccgagt tgggtccac cttggacaca	420
ctgcagctgg acgtcgccga ctttgccacc accatctggc agcagatgga agaactggga	480
atggcccctg ccctgcagcc caccagggt gccatgccgg ccttcgcctc tgctttccag	540
cgccgggcag gaggggtcct agttgcctcc catctgcaga gcttcctgga ggtgtcgtac	600
cgcgttctac gccaccttgc ccagcccga tccggtggcg gttccggtgg aggcggaagc	660
ggcgggtggag gatcagagtc caaatatggt ccccatgcc caccatgccc agcacctgag	720
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aagagcaggt ggcaggaggg gaatgtcttc tcatgctccg tgatgcatga ggctctgcac 1320  
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<210> 20

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma4 with a 30-amino acid leader peptide (Figure 2 B)

<400> 20

Met Ala Gly Pro Ala Thr Gln Ser Pro Met Lys Leu Met Ala Leu Gln  
1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro  
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu  
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys  
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu  
65 70 75 80

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser  
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu  
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105

110

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu  
 115 120 125

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala  
 130 135 140

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu  
 145 150 155 160

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg  
 165 170 175

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu  
 180 185 190

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly  
 195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ser Lys Tyr  
 210 215 220

Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Gly Pro  
 225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
 245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp  
 260 265 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val  
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys  
 325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 340 345 350

Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr  
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys  
 405 410 415

Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
 420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly  
 435 440 445

Lys

<210> 21

<211> 1365

<212> DNA

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma1 (Figure 2C)

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 atggcccctg ccctgcagcc caccagggt gccatgccgg ccttcgcctc tgctttccag 540  
 cgccgggcag gaggggtcct agttgcctcc catctgcaga gcttcctgga ggtgtcgtac 600

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aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1320  
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<210> 22

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<212> PRT

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma1 with a 30-amino acid leader peptide (Figure 2 C)

<400> 22

Met Ala Gly Pro Ala Thr Gln Ser Pro Met Lys Leu Met Ala Leu Gln  
1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro  
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu  
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys  
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu  
65 70 75 80

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Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser  
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu  
100 105 110

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu  
115 120 125

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala  
130 135 140

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu  
145 150 155 160

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg  
165 170 175

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu  
180 185 190

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly  
195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His  
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Val Ala Gly Gly Pro Ser Val  
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr  
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu  
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys  
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser  
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys  
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile  
325 330 335

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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro  
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu  
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser  
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu  
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

<210> 23

<211> 16

<212> PRT

<213> Artificial sequence, 16-amino acid peptide linker

<400> 23

Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 24

<211> 15

<212> PRT

<213> Human IgG1 hinge sequence

<400> 24

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1 5 10 15

<210> 25

<211> 10

<212> PRT

<213> Truncated human IgG1 hinge sequence

<400> 25

Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1 5 10

<210> 26

<211> 232

<212> PRT

<213> Human IgG1 Fc with native hinge, CH2 and CH3 domains

<400> 26

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
130 135 140



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Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys  
225 230

<210> 27

<211> 228

<212> PRT

<213> Human IgG2 Fc with native hinge, CH2 and CH3 domains

<400> 27

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val  
1 5 10 15

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
35 40 45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu  
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr  
65 70 75 80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn  
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro  
100 105 110

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Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln  
115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
165 170 175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
210 215 220

Ser Pro Gly Lys  
225

<210> 28

<211> 229

<212> PRT

<213> Human IgG4 Fc with native hinge, CH2 and CH3 domains

<400> 28

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe  
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser  
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser  
100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln  
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Leu Gly Lys  
225